REMARKS

Claims 25, 27-32, 34, and 39-44 are active. No amendments have been made and a clean copy of the claims is provided for the convenience of the Examiner. Favorable consideration of the remarks below and allowance of this application is now respectfully requested.

Restriction/Election

The Applicants previously elected with traverse, Group I, Claims 1-5, directed to products (microorganisms). The Applicants thank Examiner Meah for previously rejoining the method claims of Group II.

Rejection—35 U.S.C. §103(a)

Claims 25, 27-32, 34 and 39-44 were rejected under 35 U.S.C. §103(a) as being unpatentable over Fotheringham, et al., U.S. Patent No. 5,728,555, and Grifantini, et al., U.S. Patent No. 5,877,003, in view of Marceau, et al., JBC 263(32):16916. The Applicants respectfully request reconsideration of this rejection since the prior art does not disclose, suggest, or provide a reasonable expectation of success for the claimed invention.

As shown by the chart below, none of the references discloses or suggests a host cell of claim 25 which has an inactivated *dadA* gene and is transformed with both a D-carbamoylase and a D-hydantoinase gene. *A fortiori*, none of these references can provide a reasonable expectation of success for the superior properties of the claimed *E. coli* strains containing D-carbamoylase and a D-hydantoinase gene, in combination with an inactive *dadA* gene. For the convenience of the Examiner, the various teachings of the cited references are shown in the table below.

Invention: claim 25	Fotheringham	Grifantini	Marceau
An isolated *Escherichia coli* strain			
having its *dadA* gene mutated or deleted	mutated *dadA*		Mutated ***dsd*** *A* (not *dadA*)
so that said gene does not encode a functional D-amino oxidase,	No D-amino oxidase activity		
wherein said strain has been transformed with and expresses a D-carbamoylase gene and		Mutant thermostable D-carbamoylase gene	
a D-hydantoinase gene.		D-hydantoinase racemase	
Utility:	The DadX and DadA enzymes form a membrane complex which is involved in the uptake and catabolism of D-alanine to pyruvate. The DadA enzyme can also deaminate other D-amino acids such as D-phenylalanine. Accordingly, in Escherichia coli cells that are involved in the overproduction of D-amino acids, it is advantageous to mutate the dadA gene in order to prevent production of the DadA enzyme.	Preparation of D-amino acids with thermostable carbamoylase.	

None of the documents above suggest the invention by indicating that *dadA* gene should be inactivated in *E. coli* that is also transformed with a D-carbamoylase and a D-hydantoinase, nor, in combination, do these documents provide a reasonable expectation of success for the superior D-amino acid yields produced by the invention.

Fotheringham stands for the idea that in some cases that inactivation of *dadA* activity can increase D-amino acid yields, but this document does not disclose host cells transformed

with D-carbamoylase and D-hydantoinase genes. It cannot provide a reasonable expectation of success for the improved properties of constructs containing a deletion or inactivation of *dadA* along with the presence of the D-carbamoylase and D-hydantoinase genes.

The unpredictability of the effects of inactivating or including various genes is disclosed in the specification. Page 3 of the present specification indicates that it was known that:

> . . .various enzymes, such as D-amino acid oxidases. . .D-amino acid dehydrogenases. . . D-amino acid aminotransferases. . .D-hydroxyamino acid dehydratases. . .and D-amino acid racemases. . .can participate in the breakdown of D-amino acids.

However, as disclosed on page 3 of the specification, "the effect to be expected on cell growth when the various enzymes are inactivated is unknown and unforeseeable (emphasis added)". For example, page 8, lines 34 *ff.* of the specification disclose that "inactivation of the gene of the D-amino acid oxidase (*dadA*) is not sufficient to reduce breakdown" while page 9 of the specification indicates that D-amino acid production is affected by the status of other genes, for example, by inactivation of D-serine hydratase.

Grifantini was relied upon for teaching recombinant microorganisms expressing D-hydantoin racemase and mutant D-carbamoylase genes from *Agrobacterium* and methods for producing a D-amino acid. However, this document does not suggest transforming an *E. coli* cell containing an inactive *dadA* gene, nor does it provide a reasonable expectation of success that inactivating *dadA* gene activity in a cell transformed with D-hydantoin racemase and mutant D-carbamoylase genes from *Agrobacterium* would increase recovery of D-amino acids. The effects of knocking out one of many specific enzymatic activities, i.e., that of *dadA*, while including D-hydantoin racemase and D-carbamoylase genes, such as those from *Agrobacterium*, were simply unknown.

Marceau was cited as teaching that inactivating the *dsdA* gene (not *dadA*) in *E. coli* decreases degradation of D-amino acids. Therefore, Marceau is non-analogous art since it also does not suggest or provide a reasonable expectation of success for the effects obtained by inactivating the *dadA* gene in *E. coli* transformed with a D-carbamoylase and D-hydantoinase gene.

Accordingly, the Applicants respectfully request the withdrawal of this rejection since the cited prior art does not disclose, suggest or provide a reasonable expectation of success for a transformed, isolated *E. coli* having the particular genetic characteristics required by the present claims.

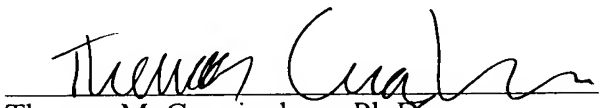
Conclusion

This application presents allowable subject matter and the Examiner is respectfully requested to pass it to issue. The Examiner is kindly invited to contact the undersigned should a further discussion of the issues or claims be helpful.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, P.C.

Norman F. Oblon



Thomas M. Cunningham, Ph.D.
Registration No. 45,394

Customer Number
22850
Tel: (703) 413 -3000
Fax: (703) 413 -2220
(OSMMN 08/03)